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AN EXPERIMENTAL ANALYSIS OF THE FACTORS GOVERNING THE HOUR OF EMERGENCE OF ADULT INSECTS FROM THEIR PUPAE

By W. N. Scott, B.Sc., B.Agr., Ph.D., D.I.C.

[Read 4th March, 1936.]

With Six Text-figures.

Contents.

		PAGE
I.	Introduction	303
II.	Historical	303
III.	Experimental	306
IV.	Discussion	324
V.	Conclusion	327
VI.	Acknowledgements	328
VII.	Literature *	328

I. Introduction.

It is well known that in many species of insects the adult emerges from the pupa at a definite time of day. The time of this emergence is fixed for each species. As this period of emergence occurs every 24 hours the factors causing it must have the same periodicity. The work recorded in this paper is an attempt to discover what factors cause this periodic emergence and their relative importance.

II. Historical.

A survey of the literature reveals that although there are records of the hour at which many adult insects have been observed to emerge from the pupa, only two workers have kept continuous records of the emergence and only one of these, Bremer, has experimented on the emergence rhythm. This lack of relevant work made it advisable to study the literature dealing with biological rhythms in general, to discover what rhythmic factors are able to cause rhythms in different forms of life and how these rhythms compare with the emergence rhythm of insects.

(a) *General Biological Rhythms.*

As records of observations and experiments on a large number of rhythms have been published it is impossible to discuss them in detail here, but the titles of all the papers consulted are given in the bibliography at the end of this paper.

The study of diurnal rhythms has attracted the greatest number of workers. The diurnal change of light and darkness is the only factor which has been proved to cause any of these rhythms, but many of them are continued under " constant " conditions. This raises the question of how the animal keeps up the rhythm when the causative factors have been removed. The existence of a " time memory " has been suggested, which enables the animal to perform certain acts at a fixed time of the day. Bohn (3) regards the cause of the descent

* *Note.*—All references marked * are taken from von Stein-Beling 1935 (25).

of *Convoluta* to be the " warning " of the shock of the approaching waves. Keeble (9, 12) does not accept this explanation but attributes the descent to the change of sign of the reaction to gravity caused by exposure to light.

That " time memory " may be the cause of the continuation of some diurnal rhythms is suggested by the fact that the animals lose the rhythm when kept for some time in " constant " conditions.

This question of " time memory " has been investigated by several workers. Forel (1910) * showed that bees which had found honey at a certain time returned at the same time next day, and von Buttel-Reepen * found that bees only visited Buck-wheat up to 10 a.m., which time corresponds to the end of nectar production by the plant. Beling (2) and Wahl * were able to train bees to pay visits to sugar at certain times of the day. They found that, when bees are trained by removing the sugar not to come after a fixed time of day, they cease to come at this time when sugar is left out for an " experimental " day. Wahl succeeded in training them to pay visits at five different times of the day, but neither he nor Beling could induce rhythms which were not associated with the 24-hour period. Beling trained bees for any hour of the day or night by keeping them in " constant " light, thus proving that the time sense is independent of light. Apparently this rhythm in bees is not connected with any periodic meteorological factor, as all known naturally recurring rhythms have been excluded.

Lutz (1934) (14) does not agree that bees have a " time memory." His experiments appeared to show that bees pay visits as long as there is a supply of nectar or sugar.

Grabensberger (1934) * says that rhythms in ants, bees, wasps and termites are associated with rhythms of metabolism. Substances which alter the metabolic rate when mixed with the food alter the rhythm. For example, arsenic in small quantities decreases oxidation and in too large quantities increases it very much. Grabensberger found that he could make the rhythm of ants early or late by increasing or decreasing the quantity of arsenic in the food, while an intermediate amount had no effect. He also found that ants differed from bees in that they could be conditioned to show rhythms with periodicities of 3–5, 21, 22, 26 and 27 hours, and they could carry on rhythms of 22 and 24 hours simultaneously.

Summing up the evidence, von Stein-Beling (25) says that it is unknown whether rhythms which are continued under " constant " conditions are due to inherited " memory " or to one individually acquired. He adds that social insects are on a higher level and probably have a " clock " regulated by the rate of metabolism in the body.

Of outstanding interest in all the experiments on diurnal rhythms is that in only one instance, namely Grabensberger's experiments with ants, was it possible to change the periodicity of the rhythm from 24 hours. This seems to indicate that the 24-hour period is a fundamental part of these rhythms.

Richter (22) and Davis (6) show that in mammals at least there are activity rhythms which are governed by the periodic functioning of internal organs.

It is doubtful whether any of these rhythms should be compared with the emergence rhythms of insects. All the rhythmic behaviour mentioned is being performed time after time by the same individual, and there is every chance of the individual " learning " by experience to perform certain actions at a fixed time. The emergence rhythm is different. Each insect emerges only once so that the individual cannot learn by experience, yet generation after generation emerges at a fixed time.

(b) *Rhythms of Emergence.*

Barnes (1) has kept records of the emergence of the different species of gall-midges with which he has worked. He summarised the results of his observations thus : " The emergence of individuals of a species extends over several hours and attains its maximum in numbers at a time which is constant for one species." The following list gives the times of emergence which he found for different species. *Dasyneura alopecuri* between 8.00 hours and 11.00 hours, *Stictodiplosis geniculati* after 9.00 hours, *Contarinia merceri* about 12.00 hours, *D. pyri* after 12.00 hours, and *Rhabdophaga terminalis* between 8.00 hours and 12.00 hours.

Barnes also carried out some experiments on the effect of light on the emergence of *D. alopecuri*. During the whole of the emergence period he kept some of his cultures in darkness from 8 p.m. until noon. This treatment causes the midges to emerge slightly later than those which are kept under normal lighting conditions; the females appear to be affected more than the males. All the cultures were kept under normal heating conditions in the insectary, but there may have been a difference in the amount of heat reaching the covered and uncovered lots.

In another experiment, one lot of cultures was kept in darkness from December to April and then put in normal light before emergence took place, while a second lot was kept in darkness the whole time. Neither treatment caused any appreciable difference from the emergence shown by the control cultures.

Bremer (5) appears to be the only worker who has carried out experiments designed to discover the cause of the diurnal rhythm of emergence shown by insects. He begins his paper by mentioning the times at which certain insects are known to emerge.

Bombyx mori emerges from 5.00 hours to 8.00 hours, *Pterogon proserpina* Pall. at sunrise, many Ephemerids between 8.00 hours and 10.00 hours, *Smerinthus tiliae* L. at 12.00 hours, *Acherontia atropos* L. between 16.00 hours and 19.00 hours, and *Cloeon dipterum* Leach between 18.00 hours and 20.00 hours. Many Butterflies and Sphingid moths emerge in the morning and many Noctuids in the evening.

Bremer suggests that the insect emerges as quickly as possible in conditions which favour its propagation, and that this time of emergence is also that at which the adult insect shows its greatest activity. He quotes figures which show that the Turnip Fly, *Pegomyia hyoscyami* Pz. and its parasites, the Ichneumon, *Phygadeuon fumator* Grav., and the Braconid, *Opius fulvicollis* Thoms., emerge in the morning, whereas the Meal Moth, *Ephestia kühniella* Zell., emerges in the evening. These times of emergence correspond with the times of the greatest activity of the imago.

He kept *E. kühniella* in the dark during the day and exposed them to the light of an electric lamp during the night. Under this treatment 60% of the insects emerged in the morning. From this he concluded that it is probable that reduction of light is an incentive for emergence from ripe pupae, but a high proportion of insects always remain which emerge in the evening in spite of " reversed " lighting conditions. Temperature was not controlled during this experiment, and he suggests that this may also have been a factor affecting the altered time of emergence.

The emergence of *E. kühniella* kept in constant darkness is almost the same as that which they show when kept under normal lighting conditions. The emergence of *O. fulvicollis* from Turnip Fly pupae kept under " reversed "

lighting conditions is slightly later than when kept under normal conditions. Bremer concludes by suggesting that probably the emergence of the adult insect is governed by some external factor which has a diurnal rhythm, but the possibility of an inherent inner factor must not be neglected. The small number of insects used in his experiments and unsatisfactory experimental conditions prevented him from arriving at a more definite conclusion.

III. Experimental.

(a) *Material Used.*

The insect used in the investigation was the Phycitid moth, *Ephestia kühniella* Z. (Mediterranean Flour Moth). In experiments at Slough it was noticed that *E. kühniella* bred in the insectary emerged in greater numbers during the evening than at any other time, even when full-grown larvae or pupae were moved into the " constant " temperature room (27° C.). Bremer (5) also found this rhythm of emergence in *E. kühniella* Z. This insect was chosen because a continuous supply of all stages can be obtained throughout the year. It is part of the routine work at the Biological Field Station, Slough, to make up a large number of cultures of *E. kühniella* Z. every week, so that material was always available. In all experiments except those in which it was necessary to have insects reared in " constant " conditions, stock reared in the insectary was used. These stock insects are reared in tins on wholemeal flour, and special precautions are taken to ensure that they remain healthy (Mansbridge, 15). Each tin contains the offspring of a single pair, a brother and sister mating. Unfortunately an attempt to use the Fruit Fly, *Drosophila melanogaster* Mg., had to be abandoned, after two experiments with it were completed, because it was impossible to take the necessary readings, as well as those of the *E. kühniella* Z. experiments, within a reasonably short time of the exact hour of observation. The *D. melanogaster* used in these two experiments were reared in glass jars on banana agar and yeast (Sturtevant, 26).

(b) *Method of Work and Apparatus.*

Both the method of work and the apparatus were varied and improved on as the work progressed so that each is dealt with in the detailed account of the experiments.

(c) *Method of Making Observations.*

The moths were removed from the cultures each time an observation was made. In the early experiments the moths were removed with a pair of forceps, but as the number of cultures in the experiments increased, this method took too much time. Putting each cocoon separately into a gelatin capsule and spreading the capsules on trays was then tried. This increased the speed with which observations could be made, but the work entailed in setting up experiments took much longer, and the risk of damaging the insects was increased. The method finally adopted was to suck up the moths into a glass specimen tube. An ordinary " sucking tube " with muslin over the outlet to prevent the insects being sucked into the mouth of the user was unsatisfactory as wing-scales and flour passed through the muslin and choked the operator. This difficulty was overcome by inserting a conical flask between the specimen tube and the mouthpiece. The flask contained about an inch of water covered by a layer of olive oil. The inlet tube from the specimen tube dipped into the water below the oil layer so that when air was sucked out of the flask air from the specimen tube bubbled through the

liquid. The reduction of surface tension at the interface between the water and the oil results in the wetting, and consequent trapping of wing-scales and dust. This apparatus was very convenient to use and moths could be removed from the cultures very quickly. It had another advantage: moths could either be counted as they were removed, or the moths from each culture could be sucked into a separate specimen tube which could then be removed and the moths counted later.

In the early experiments observations were made at hourly intervals from 10.00 hours to 19.00 hours and again at 22.00 hours. In experiments on the effect of light observations were made every two hours. It was hoped that the effect of the light necessary for making observations would be reduced by using this longer interval. When it became apparent that it was necessary to make observations at equal intervals throughout experiments, an interval of four hours was chosen. Perhaps a period of four hours between observations is longer than seems to be advisable in this work, but it is the shortest that could be continued throughout an experiment and yet allow the observer to have some sleep.

(d) *Method of Presenting Results.*

The experiments are presented in this paper in the order that was thought to be most suitable and not in the order in which they were actually carried out.

In every experiment each culture of insects was kept separate and the emergence of moths from each was noted.

The emergences from each culture are not given separately but have been added when all the cultures in an experiment showed the same result.

With four exceptions the emergences observed at the same hour of each day have been added. The resulting table shows the relationship between the emergence rhythm and the diurnal period. In experiments 12 and 13 the observations were added in periods of 36 hours and 16 hours respectively, and in two other experiments all the observations have been given to demonstrate points of interest.

The emergence rhythm together with the temperature rhythm is shown in graphical form for some experiments. These graphs give an excellent representation of the emergence rhythm and its relationship with the temperature rhythm.

Before it was realised that observations should be made at regular intervals throughout experiments, a number of readings were missed on different days. Since it is impossible to tell when the emergences counted at the next observation had occurred, all readings made on these days have been omitted. The only effect of these omissions is to reduce the total number of emergences observed in the experiment, because the rhythm investigated has a diurnal periodicity. Further, the observations which were made on these days agree with those made at corresponding times on other days, and when some of the experiments were repeated with no omissions, the results obtained confirm this opinion.

To prevent confusion the 24-hour " clock " has been used to indicate the time of day. Corrections have been made for " summer time," *i.e.* sun time was used.

A statistical investigation of experiment 7 was thought desirable. " Periodogram Analysis " and " Goodness of Fit " tests were used, but opinion differs as to whether or not these are strictly applicable.

There are two objections to the application of "Periodogram Analysis" to this work. A "series" to which periodogram analysis can be applied must have the form of the "sine curve." The emergence rhythm in the constant temperature room has this form but does not have it in the insectary, where the moths emerge only during a very short period of each day. The other and perhaps more important objection is that "Periodogram Analysis" can only be applied to a "constant series." The diurnal emergence rhythm is not a "constant series," as many more emergences occur during the days in the middle of experiments than during the days at the beginning and end.

The application of the "Goodness of Fit" test is also doubtful. The series composed of the total emergence between each time of observation during the day can be compared with a series composed of the mean of these totals by the $\chi^2 = S\frac{x^2}{m}$ formula. This series composed of the mean has no rhythm as the figures are in the proportion of 1 : 1 : 1 : 1 : 1 : 1. If the observed series differs significantly from this and appears to have a rhythm it may be said to have one.

(e) *Experiments on the Effect of Temperature.*

Experiment 1.

Observations on the rhythm of emergence shown by *E. kühniella* Z. in the insectary.

Method.—The pupae from six cultures of stock reared in the insectary were put separately into gelatin capsules and placed on trays. The pupae from each culture were kept separate and the trays were placed on a shelf in the insectary. Observations were made every hour from 10.00 hours to 19.00 hours and again at 22.00 hours.

Result.—536 emergences were observed and recorded in Table I. This experiment shows that there is a very definite rhythm of emergence in the insectary with a maximum between 17.00 hours and 18.00 hours. The cultures used in this experiment had been subjected to the temperature rhythm of the insectary from the time the eggs were laid, about the middle of August, until the insectary was heated at the end of September.

No record of the temperature variation in the insectary was made during this period, but it is probable that its rhythm closely followed that of the laboratory, where the maximum temperature occurred at 16.00–18.00 hours. After the insectary was heated, observations made during the experiment showed that the temperature remained constant during the day but fell in the evening and was probably at its lowest about 6.00 hours.

Table I.

Time between observations	22–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–22
Number of emergences	156	2	7	1	2	1	16	10	238	30	73

The cocoons were exposed to the dim light coming through the insectary window, from the time when they were put into the capsules. At the time of year when the experiment was being done darkness fell at 17.00 hours.

The observed peak of emergence may have been caused either by light or the temperature factor acting separately or by their combined effect.

Experiment 2.

To confirm Experiment 1.

Method.—The emergence of moths from four tins of insectary stock were recorded. The cultures were kept in the breeding-tins in order to leave the pupae undisturbed.

A thermograph was kept with the tins during the last seven days of the experiment. Observations were made every four hours for a period of 17 days.

Result.—The emergences observed are given in Table II and the relationship of the emergence rhythm to the temperature rhythm is shown on Graph I.

TABLE II.

Time between observations	22–2	2–6	6–10	10–14	14–18	18–22
Number of emergences	78	8	0	2	116	631

This experiment confirms the result obtained by Experiment 1, although the maximum emergence is a little later. It is impossible to say how much later because of the times at which observations were made, but it was not more than four hours. It is probable that it was less than this, when the number of emergences observed at 18.00 hours and 2.00 hours is considered.

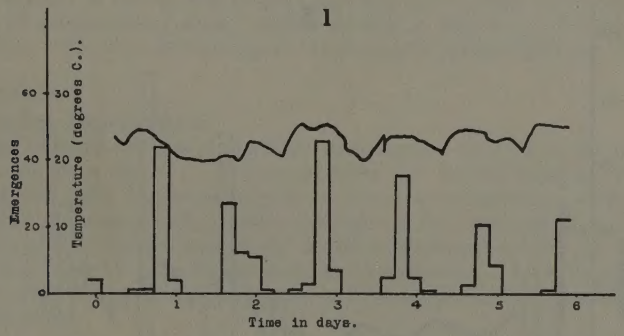

FIG. 1.—The relationship between emergence and temperature rhythms.

It will be noticed from Graph I that the maximum emergence occurs when the temperature begins to fall.

The same result was obtained from Experiment 10 (Graph III) and from six cultures used as a control in Experiment 17 (Graph II). In these experiments the light factor has been eliminated, so it appears that the rhythm of emergence is caused by the diurnal rhythm of temperature. Graphs I and II show that the maximum emergence occurs when the temperature begins to fall.

Experiment 3.

Is the rhythm of emergence continued when pupae from insectary-reared stock are kept at a constant temperature?

Method.—The cocoons from 9 cultures of insectary-reared material were put into glass jars covered with muslin. These jars were put in the constant-temperature room (27° C.). The control halves of 6 cultures in Experiment 8

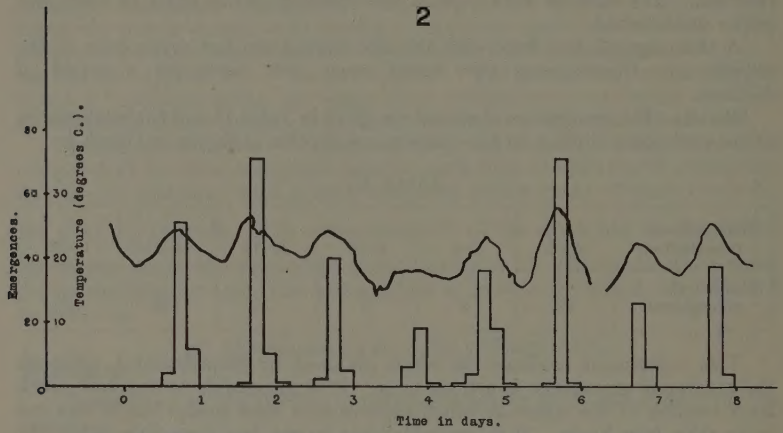

FIG. 2.—The relationship between emergence and temperature rhythms.

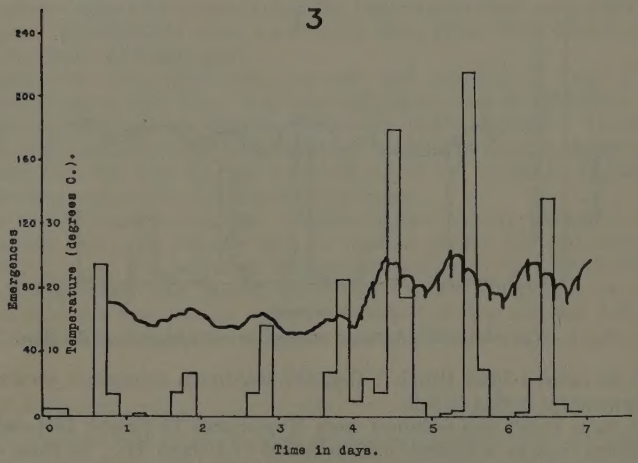

FIG. 3.—The change in the time of maximum emergence when the time of maximum temperature is changed.

were treated in a similar manner and the results observed are given here. Hourly observations were made from 10.00 hours to 19.00 hours.

Result.—1335 emergences from these cultures were observed and tabulated (Table III).

The greatest number of moths emerged between 19.00 hours and 10.00

hours. The average number of moths per hour between these times is much greater than for any hour during which observations were made. There must be a period of greatest emergence between 19.00 hours and 10.00 hours, as it seems unlikely that the emergence should be the same for each hour between these times and yet be much greater than for any hour between 10.00 hours and 19.00 hours.

TABLE III.

Time between observations	19–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19
Number of emergences	979	47	48	57	45	27	23	43	26	40

Table III also shows that there is a smaller peak of emergence between 10.00 hours and 13.00 hours. This may be the result of a stimulation by shock when the cultures were handled at 10.00 hours after they had been untouched for 15 hours. The cocoons were also exposed to light for varying lengths of time during the day, owing to the number of people working in the constant-temperature room.

Even making allowances for these factors, it seems that *E. kühniella* Z. reared in the insectary retain an emergence rhythm which causes them to emerge in greatest numbers between 19.00 hours and 10.00 hours, when they are moved into a constant temperature.

This rhythm is not as well defined as when the cultures are exposed to a rhythm of temperature, many moths emerging between 10.00 hours and 14.00 hours, while very few emerge in the insectary during this time.

Experiment 4.

To confirm Experiment 3.

Method.—Eight cultures of insectary-reared pupae were put in the constant-temperature room (27° C.). The pupae were left in the breeding tins, thus excluding the light factor mentioned in the last experiment. Observations were made at intervals of 4 hours throughout the experiment so that the disturbing of the cultures was evenly distributed throughout the day.

Result.—Table IV gives the result of this experiment. As in Experiment 3 there is a definite rhythm of emergence with a maximum between 18.00 hours and 22.00 hours.

TABLE IV.

Time between observations	22–2	2–6	6–10	10–14	14–18	18–22
Number of emergences	324	257	175	198	332	456

This experiment was done at the same time as Experiment 2, and the maximum emergence in both occurs at the same time. The rhythm was not as definite in the constant-temperature room as in the insectary.

All the cultures in this experiment did not have a maximum emergence between 18.00 hours and 22.00 hours. One culture showed a maximum emergence between 14.00 hours and 18.00 hours and another culture between

22.00 hours and 2.00 hours. As a result the magnitude of the rhythm of emergence is really greater than the table indicates.

Experiment 5.

The study of the emergence of moths from cultures which have been kept in a constant-temperature from the first larval instar.

Method.—10 large glass sweet jars, covered with muslin, each containing 100 larvae in the first instar, were put in the constant-temperature room. When the moths began to emerge observations were made every hour from 10.00 hours to 19.00 hours and again at 22.00 hours.

Result.—The emergence of moths is given in Table V. There is a very distinct rhythm of emergence with a maximum between 12.00 hours and 22.00 hours. This rhythm may have been caused by a temperature rhythm to which the larvae were subjected before they were put into the constant-temperature room. The light effects, which have been mentioned in previous experiments, may also have been a partial cause of this rhythm.

TABLE V.

Time between observations	22–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–22
Number of emergences	146	26	6	13	24	33	19	36	36	40	108

Experiment 6.

Do moths which have been in a constant temperature during their whole life cycle show a rhythm of emergence?

Method.—Moths which had emerged in the constant-temperature room (27° C.) were paired. Each pair was put into a large glass jar with whole-meal flour as food for the larvae. These jars were covered with muslin. Observations were made every hour from 10.00 hours to 19.00 hours and again at 22.00 hours.

Result.—Table VI shows that most of the moths emerged between 15.00 hours and 22.00 hours and that there was a period of greatest emergence between 15.00 hours and 19.00 hours. This rhythm cannot be caused by any direct effect of temperature, as the parent moths had been in the constant-temperature room from the pupal stage. It is possible that the rhythm might have been impressed on the immature gonads of the parent while they were still in the larval stage, before they were put into the constant-temperature room.

TABLE VI.

Time between observations	22–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–22
Number of emergences	144	11	8	11	12	18	43	46	33	34	74

Experiment 7.

To confirm Experiments 5 and 6.

Method.—Eight tins containing larvae were moved from the insectary to the constant-temperature room. The moths from these cultures began to

emerge after three weeks. Two of these cultures were selected because they appeared to be more healthy than the other cultures and more moths were emerging from them. These two cultures were used to breed a second generation. At each observation the moths which had emerged from these cultures were put into specimen tubes. When any of these moths were seen to pair the female was kept and, if she laid a number of eggs, a culture was started with them. In this manner 23 new cultures were started. 17 of these were from one parent culture and the moths used had emerged as follows : 7 pairs between 22.00 hours and 2.00 hours, 4 pairs between 2.00 hours and 6.00 hours, 3 pairs between 10.00 hours and 14.00 hours, 2 pairs between 14.00 hours and 18.00 hours and 1 pair between 18.00 hours and 22.00 hours. Six were from the other parent culture and had emerged as follows : 2 pairs between 2.00 hours and 6.00 hours, 2 pairs between 6.00 hours and 10.00 hours, 1 pair between 14.00 hours and 18.00 hours and 1 pair between 18.00 hours and 22.00 hours.

When the moths from these cultures emerged the same method was employed to breed a third generation. This third generation differed from the second generation in that moths taken from all the 23 cultures were used. Even by doing this it was only possible to raise twelve cultures in the third generation and comparatively few moths were obtained from these. This was due to the stock becoming sterile because of the high temperature (27° C.) at which it was kept (Norris (18)). Observations were made every four hours.

Result.—Table VII gives the result of this experiment. The emergence from each culture of the first generation is given. These cultures showed much greater variation than those in Experiment 4 in which the insects were subjected to the same treatment. The number of emergences from some of the cultures is small and little significance can be attached to them. The emergence from all the cultures showed a rhythm with a maximum between 18.00 hours and 6.00 hours. The rhythm is small, but this is partly due to the maximum emergence from all the cultures not having occurred at the same time.

When tested by periodogram analysis the rhythm showed a periodicity of 28 hours, although when the cultures were tested separately all but two showed a periodicity of 24 hours, and these show no rhythm. Probably the 28-hour periodicity shown by the analysis is caused by the variation in time of the maximum emergence shown by different cultures.

The rhythm was also tested for " goodness of fit " to a series in the proportion of 1 : 1 : 1 and was found to differ significantly from it. It was fortunate that the cultures from which the second generation was bred were " D," which showed most rhythm, and " C," which showed least. The emergences observed from each culture are not given separately, as the time at which the individual parents emerged had no effect on the time of the maximum emergence of their offspring. The total emergence for the descendants of " D " and " C " are given separately. Both lots of cultures showed the same emergence rhythm, so that the strength of the rhythm shown by the parent culture did not affect the rhythm shown by the offspring.

The second generation showed a greater rhythm of emergence than the first. The third generation also shows a definite emergence rhythm. The rhythms of both these generations were treated by periodogram analysis and found to have a periodicity of 24 hours. They were also tested for " goodness of fit " and were found to differ significantly from a series in the proportion of 1 : 1 : 1.

TABLE VII.

Time between emergences	22–2	2–6	6–10	10–14	14–18	18–22
First Generation.						
Number of emergences from Culture A	37	42	26	20	23	20
,, B	20	22	14	13	15	24
,, C	32	41	38	27	44	36
,, D	90	70	38	29	29	91
,, E	12	17	8	18	17	17
,, F	2	6	1	7	11	6
,, G	12	7	12	9	13	19
,, H	18	17	22	35	32	25
Total	223	222	159	158	184	238
Second Generation.						
Number of emergences from offspring of Culture D	520	398	294	320	367	445
,, C	115	133	58	46	67	126
Total	635	531	352	366	434	571
Third Generation.						
Number of emergences	154	196	114	106	142	145

Experiment 8.

The effect on the emergence rhythm of keeping pupae at a low temperature and moving them to a high temperature for 2 hours each day.

Method.—Six cultures of insectary stock were halved and the pupae put into glass jars covered with muslin. One lot of half-cultures was put in the constant-temperature room (27° C.). The other lot was put in a cool incubator (14° C.) and moved to the constant-temperature room for 2 hours (10.00 hours to 12.00 hours) daily. Observations were made every hour from 10.00 hours to 19.00 hours and during the last month of the experiment again at 22.00 hours.

Result.—Because of the higher temperature the insects in the constant-temperature room emerged during a much shorter period (23rd October to 3rd November) than those in the cool incubator (23rd October to 3rd December). The emergence from both lots is given in Table VIII. The control insects kept in the constant-temperature room emerged throughout the 24 hours with a maximum emergence during the night. Those in the cool incubator had a period of maximum emergence between 14.00 hours and 15.00 hours, the emergence gradually decreasing to 19.00 hours. The emergences shown in the table as having occurred between 19.00 hours and 10.00 hours may be assumed to have occurred between 19.00 hours and 22.00 hours, because during the last month of the experiment when observations were made at 22.00 hours only 3 moths emerged between this hour and 10.00 hours.

TABLE VIII.

Time between observations	19–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19
Number of emergences in										
C.-T. Room	228	9	12	9	4	3	10	7	11	5
Cool Incubator	106	1	11	2	2	78	32	30	39	28

The change from low to high temperature at 10.00 hours appears to have a slight immediate effect, as a few moths emerged before 12.00 hours. The greatest effect of the treatment took place two hours after the change from the high to the low temperature. In this experiment the maximum emergence was made to occur about 4 hours earlier than it does in the insectary.

Experiment 9.

The alternative experiment of keeping pupae at a high temperature and moving them to a low temperature for 2 hours each day was also performed.

Result.—As may be seen from Table IX the number of emergences was small. The only effect of the treatment was to retard the time of emergence.

TABLE IX.

Time between observations	22–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–22
Number of emergences											
at 27° C.	12	–	–	–	3	4	5	11	11	12	36
„ 27 & 14° C.	33	–	–	–	–	–	–	1	2	3	33

Experiment 10.

To discover if the time of maximum emergence can be changed by changing the time of maximum temperature.

Method and Apparatus.—An incubator was constructed in which the temperature could be made to have a rhythmic variation. One end of a 50-gallon metal oil-drum was removed. A 10-gallon metal drum was suspended inside the large one. A collar of plywood formed a lid for the space between the drums and plywood lids were fitted to both drums.

The space between the drums was filled with water and a bunsen burner was put beneath the drums. By regulating the bunsen flame and the quantity of water it was found that the temperature inside the smaller drum rose and fell about the same number of degrees when the bunsen was kept alight for a fixed period in each 24 hours. This water-jacketed incubator was very convenient and gave a regular rhythm of temperature which could be adjusted as required for time and for length of period. However, it had one disadvantage. The maximum and minimum temperatures were of necessity higher than those in the laboratory or insectary and in consequence, when the incubator was opened to count the moths, the temperature fell abruptly, especially at the higher temperatures. However, it rose again immediately the lid was replaced. The fall in temperature in the cultures was not as great as shown on the thermograph chart, for the thermograph was removed during the

whole time necessary to make an observation, while each tin was returned to the incubator immediately the moths were removed from it.

As the tins in which the insects were reared were too big to put into the incubator the pupae from 8 cultures were moved into smaller tins, 6″ in diameter and 4½″ high. These cultures were kept in the insectary for 4 days and then moved into the incubator. The bunsen burner was lighted at 22.00 hours and turned out at 6.00 hours each day. Observations were made at 4-hour intervals.

Result.—The number of moths which had emerged at each observation is given on Graph III together with the temperature recorded by the thermograph. The emergences are also given in Table X. This table shows at once that the maximum emergence was about 8 hours earlier in the incubator than in the insectary. A study of Graph III shows that the maximum emergence occurs soon after the temperature has reached a maximum and has begun to fall. When the time of maximum temperature is changed the time of greatest emergence is changed by the same amount.

TABLE X.

Time between observations	22–2	2–6	6–10	10–14	14–18	18–22
Number of emergences in:						
insectary	4	5	0	0	152	183
incubator	9	25	41	523	110	10

Experiment 11.

To confirm Experiment 10 and to discover if the new time of maximum emergence is continued in constant temperature.

Method.—The same apparatus was used as in Experiment 10. Eight cultures of insectary stock were put in small tins. These were kept in the insectary for 24 hours before they were moved to the incubator. After 5 days in the incubator the cultures were moved to the constant-temperature room (27° C.). A thermograph was kept with the cultures when in the incubator. Observations were made every four hours.

Result.—The number of emergences at each observation is given on Graph IV, together with temperature recorded by the thermograph. Table XI also gives the number of emergences. This experiment confirms the result obtained from Experiment 10. The time of maximum emergence is changed in the incubator so that it occurs shortly after the temperature begins to fall. This experiment also shows that the emergence rhythm with the new time of maximum emergence is continued in constant temperature, but the period of greatest emergence is lengthened when the temperature which caused the rhythm is removed.

The number of moths which emerged in the constant-temperature room is smaller than in the incubator, because most of the insects in the cultures had already emerged. A point of interest in this experiment is that the time of maximum temperature was advanced successively on the last two days in the incubator. This advance was shown immediately by the emerging moths. Also the time of maximum emergence in the constant-temperature room was the same as that on the last day in the incubator, although the cultures had

only once been subjected to the temperature rhythm which gives the maximum emergence at this time. Somewhat similar results were obtained by Johnson (11) with the activity rhythm of deer mice, by Lutz (13) with the activity rhythm of crickets and by Schleip (23) with the rhythm of colour change in stick insects. When these rhythms were "reversed" experimentally the "reversed" rhythms continued in constant conditions.

TABLE XI.

Time between observations	22–2	2–6	6–10	10–14	14–18	18–22
Number of emergences in the insectary: 4th July	4	0	0	0	45	135
Number of emergences in the incubator:						
5th July	17	6	0	69	176	32
6th ,,	2	3	8	157	102	12
7th ,,	0	0	1	166	37	0
8th ,,	0	0	54	126	20	4
9th ,,	1	4	108	37	0	0
Total	20	13	171	555	335	48
Number of emergences in the C.-T. Room:						
10th July	1	26	87	3	2	0
11th ,,	0	12	50	12	2	1
12th ,,	1	24	23	16	8	2
Total	2	62	160	31	12	3

Experiment 12.

To discover if a rhythm of emergence with a periodicity longer than 24 hours can be induced by lengthening the periodicity of the temperature rhythm.

Method.—Eight cultures of insectary stock were treated as in the last experiment. The bunsen burner was kept alight for periods of 12 hours alternating with periods of 24 hours when it was turned off. This gave a 36-hour temperature rhythm in the incubator. Observations were made every four hours.

Result.—Graph V gives the emergences together with the temperature record for the experiment. There is a rhythm of emergence with a maximum every 36 hours, shortly after the temperature begins to fall. These peaks of emergence have been marked "P" on the graph. Between these peaks are others marked "S," which occur when the temperature is rising. These secondary peaks of emergence occurred approximately 24 hours after the primary peaks.

Experiment 13.

To discover if the diurnal rhythm of emergence can be altered by a temperature rhythm with a periodicity of less than 24 hours.

Method.—Eight cultures of insectary-reared stock were treated as in

Experiment 11. The incubator was adjusted to have a rhythm of temperature with a periodicity of 16 hours by keeping the bunsen burner alight for periods of 4 hours, alternating with periods of 12 hours when it was extinguished. Observations were made every four hours.

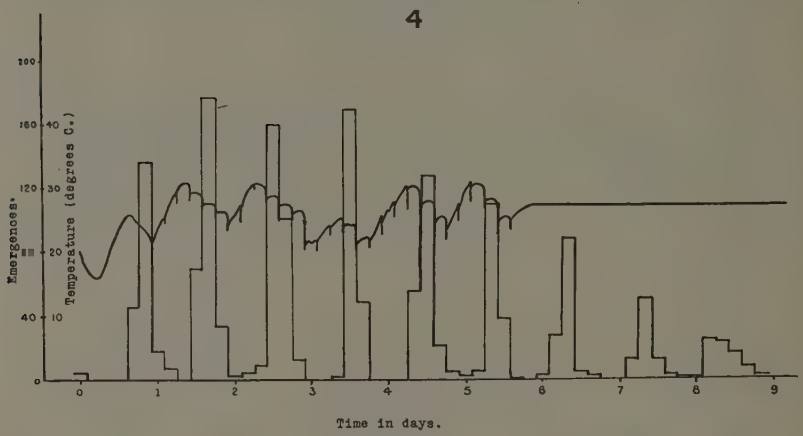

Fig. 4.—The time of maximum emergence.

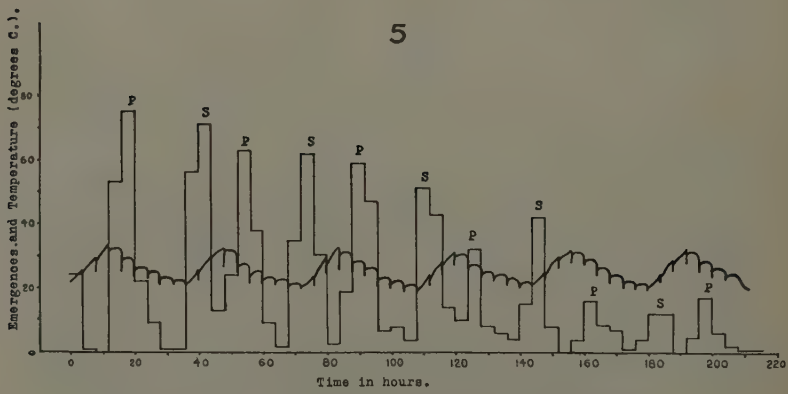

Fig. 5.—The attempt to produce a 36 hour rhythm of emergence.

Result.—Graph VI shows that an emergence rhythm with a periodicity of 16 hours can be induced. As in other experiments the maximum emergence occurred while the temperature was falling.

Experiment 14.

To show the effect of light on the emergence rhythm.

Method.—The cocoons from six cultures of insectary stock were put into

capsules. Each culture was divided in half and the capsules put on separate trays. The trays were put on a table in a cellar, one lot of half-cultures at each end. An electric light (75-watt lamp) was fixed over the centre of the table and three feet above it. This lamp was kept alight during the whole of the experiment. The trays at one end of the table were covered with a light-proof box from 10.00 hours to 22.00 hours, and those at the other end were covered from 22.00 hours to 10.00 hours.

A record of the temperature in the cellar was kept. It showed a slight

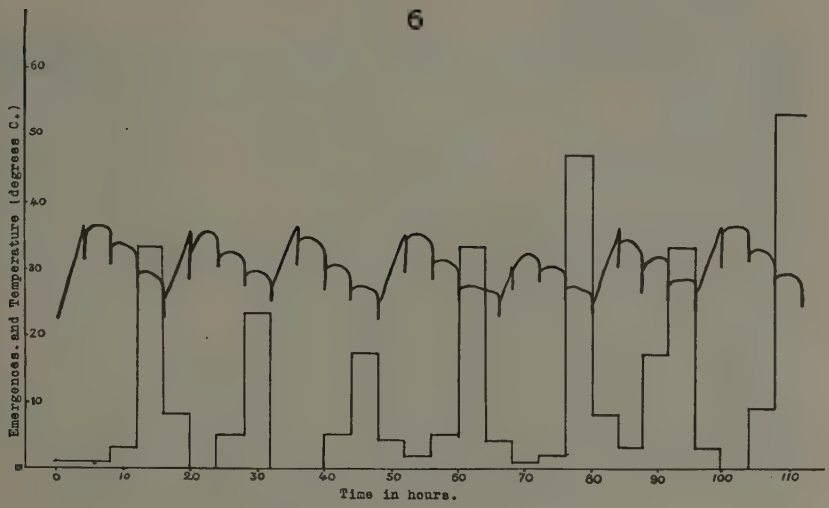

FIG. 6.—The 16 hour rhythm of emergence.

rhythm, 19°–20° C. from 14.00 hours to 22.00 hours, which dropped to 17°–18° C. at 8.00 hours.

A thermometer was also kept with each set of trays. These thermometers, at times, showed a difference of one or two degrees, but there seemed to be no regularity in these differences. Observations were made every two hours from 10.00 hours to 18.00 hours and again at 22.00 hours.

TABLE XII.

Time between observations	22–10	10–12	12–14	14–16	16–18	18–22
Number of emergences covered 22–10 hours	116	0	0	1	3	176
10–22 ,,	197	94	11	8	4	8

Result.—Table XII gives the emergence in this experiment. The moths emerged immediately before covering and during the time when they were covered, from the lot which was covered at 22.00 hours. In the lot covered at 10.00 hours few moths emerged more than 2 hours after covering. It is unfortunate that the covering and uncovering was done at 10.00 hours and

22.00 hours, because no observations were made when one lot was exposed to light and the other was covered.

It is evident, however, that the different treatments caused a difference in the emergence rhythms, but it is impossible to say whether this was due to the light factor or to the heating effect of the lamp, though the latter must have been very slight.

Experiment 15.

To confirm Experiment 14.

Method.—Eight cultures of insectary stock were treated as in Experiment 14, except that instead of being covered with boxes which had to be removed by hand, two boxes were fitted with lids which could be removed automatically at any set time. This apparatus consisted of two boxes, $2'\ 2\frac{3}{4}'' \times 1'\ 9'' \times 3\frac{1}{4}''$, fastened to the table, one at each end. These boxes had movable lids, felted round the edges to make them light-proof. Each lid was kept closed by a slat of wood, held across it by a wire trigger. A "break-back" mouse-trap, which was released by the striker of an alarm clock, struck against the trigger and released the lid. The lid was lifted clear by elastic fastened to the ceiling.

One lot of pupae was exposed to the light from 0.00 hours to 12.00 hours and the other from 4.00 hours to 16.00 hours, so that observations could be made before and after covering. Observations were made every 2 hours from 8.00 hours to 18.00 hours and again at 22.00 hours.

Result.—Table XIII shows that both lots had a period of maximum emergence after covering, but in contrast to the last experiment they did not show a peak of emergence in the preceding 2 hours, although there is an increase in the numbers which emerged during this period. It seems probable that there were temperature changes correlated with the changes of light, therefore it is impossible to say whether the emergence is produced by the change from light to darkness or not. On two days the lamp went out and on these days the maximum emergence was observed at the next inspection after this occurred. These accidents prove that the lamp caused the rhythm. (The observations made on these days are not included in Table XIII.)

Table XIII.

Time between observations	22–8	8–10	10–12	12–14	14–16	16–18	18–22
Number of emergences from lot covered at 12.00 hours	90	33	11	126	16	11	9
" " 16.00 "	10	12	11	20	38	211	27

Experiment 16.

To confirm the result obtained from Experiment 15.

Method.—The pupae still in their cocoons from 9 cultures of insectary stock were divided into 3 lots "A," "B" and "C" and put into glass tumblers, the tops of which were covered with muslin. The tumblers were put on the concrete floor of an insectary. Lot "B" was left uncovered. At 13.00 hours each day lot "A" was covered with a wooden box, round the edges of which felt had been fixed to make it light-proof. This box was removed again at 1.00 hour. Lot "C" was kept under a similar box all the time. A

thermograph was kept on the floor with the cultures. Observations were made every four hours.

Result.—The emergences observed during this experiment are given in Table XIV. The majority of moths in lot " A " emerged between 13.00 hours and 21.00 hours. A slightly greater number emerged between 13.00 hours and 17.00 hours than between 17.00 hours and 21.00 hours, which suggests that the maximum emergence was immediately before 17.00 hours. The moths from lots " B " and " C " emerged between 13.00 hours and 1.00 hour.

TABLE XIV.

Time between observations	21–1	1–5	5–9	9–13	13–17	17–21
Number of emergences from lot A	34	8	1	8	168	120
,, B	23	5	1	5	50	292
,, C	75	17	1	2	46	209

This experiment shows that the change from light to darkness causes the moths to emerge. The result obtained from the next experiment, 17, contradicts this. Another explanation of the emergence shown in this experiment can be given. The cultures were on the concrete floor of an insectary which was rather cold. When lot " A " was covered at 13.00 hours the temperature had not attained its maximum, which was shown by the thermograph to have occurred at about 17.00 hours. It seems probable that when the box was put over lot " A " the temperature did not rise further but immediately began to fall, because the volume of air was small and in contact with the cold floor. Of course the glasses came in contact with air at a higher temperature when the subsequent observation was made at 17.00 hours, but it is unlikely that the rise penetrated into the cultures during the short time necessary to make an observation.

Therefore it appears that the difference in emergence shown by lot " A " and lots " B " and " C " is due to temperature effects and not to light effects.

Experiment 17.

The effect of light on the emergence rhythm.

Method.—Eighteen tins of insectary stock were divided into 3 lots of 6 tins each and put on a table in a room where there was strong daylight. The lids were kept on the tins in lot " A." In lot " B " the lids were removed at 4.00 hours (later at 3.00 hours), and pieces of glass were substituted for them so that light could reach the cultures at the bottom of the tins. The lids were put on again at 12.00 hours (later 11.00 hours). In lot " C " glass was substituted for the lids from 4.00 hours until 20.00 hours (later 3.00 to 19.00 hours). A thermograph was kept on the table with the cultures. Observations were made every four hours. After 3 days the observations were made an hour earlier. On the last day of the experiment (29th July) lots " A " and " B " were uncovered from 3.00 hours until 19.00 hours. Lot " C " was covered during the whole of the last day.

Result.—Three days after the experiment had been started it was noticed that there was only a slight difference in the periodicities of the emergence rhythms shown by the three lots of cultures. In lot " C " no moths had emerged before 16.00 hours when they were covered, while in lots "A " and

" B " a few moths (7 in " A " and 21 in " B ") had emerged at this time. These numbers are so small that they might have been due to chance. If this was not so and they were influenced by the experimental conditions, it appears that in lots " A " and " B " the moths had commenced to emerge before 16.00 hours and that possibly the greatest emergence was immediately after this hour. It was also possible that the moths in lot " C " did not begin to emerge until much later, and that the greatest number of emergences occurred immediately before 20.00 hours. If light is a factor inhibiting emergence it is probable that its power of inhibition depends on its intensity and, as at 20.00 hours in July the light has already faded perceptibly, it was thought that the observations were not being taken at the proper times to show the effect. Accordingly the times of making the observations were advanced by one hour.

From the emergences then recorded it is clear that the deductions drawn from the observations made on the first three days are correct. The maximum emergence from lots " A " and " B " was between 15.00 hours and 19.00 hours, and the greatest emergence from lot " C " was between 19·00 hours and 23.00 hours.

The observations made during this experiment are given in detail in Table XV. Graph II shows the relationship between the emergence rhythm of lot " A " and the temperature rhythm.

On 28th July at 19.00 hours, it was noticed that the wings of 90% of the moths which had emerged from lots " A " and " B " were dry. This showed that they had emerged some time before. 70% of the moths which had emerged from lot " C " had wet wings, indicating that they had just emerged.

There was no difference in the time of maximum emergence shown by lots " A " and " B."

The observations made on 29th July are very interesting. On this day lot " C " was covered all the time and the majority of the moths emerged

TABLE XV.

	Emergence from lot " A."					
Times between observations	0–4	4–8	8–12	12–16	16–20	20–24
21st July	0	0	0	4	51	11
22nd ,,	0	0	0	1	71	10
23rd ,,	1	0	0	2	40	5
Total	1	0	0	7	162	26
Times between observations	23–3	3–7	7–11	11–15	15–19	19–23
24th July	0	0	0	0	6	18
25th ,,	1	0	1	4	36	18
26th ,,	1	0	0	1	71	1
27th ,,	0	0	0	0	26	6
28th ,,	0	0	0	0	37	4
Total	2	0	1	5	176	47
29th July	1	0	0	0	39	5

TABLE XV (*continued*).

Emergence from lot "B."						
Times between observations	0–4	4–8	8–12	12–16	16–20	20–24
21st July	0	0	0	7	34	14
22nd ,,	1	0	0	1	43	5
23rd ,,	1	0	0	3	37	6
Total	2	0	0	11	114	25
Times between observations	23–3	3–7	7–11	11–15	15–19	19–23
24th July	0	0	0	0	5	7
25th ,,	1	0	0	2	26	2
26th ,,	0	0	0	1	51	3
27th ,,	0	0	0	0	27	10
28th ,,	0	0	0	0	47	6
Total	1	0	0	3	156	28
29th July	0	0	0	0	43	39
Emergence from lot "C."						
Times between observations	0–4	4–8	8–12	12–16	16–20	20–24
21st July	0	0	0	0	20	9
22nd ,,	0	0	0	0	40	8
23rd ,,	3	2	0	0	41	2
Total	3	2	0	0	101	19
Times between observations	23–3	3–7	7–11	11–15	15–19	19–23
24th July	0	0	0	0	4	18
25th ,,	0	0	0	0	13	39
26th ,,	0	0	0	0	36	40
27th ,,	0	0	0	0	6	55
28th ,,	0	0	0	0	29	72
Total	0	0	0	0	88	224
29th July	2	1	0	0	73	12

between 15.00 hours and 19.00 hours. Lots "A" and "B" were exposed to light until 19.00 hours. Most of the moths from lot "A" emerged between 15.00 hours and 19.00 hours, while from lot "B" almost 50% emerged between the same times. If light is an inhibitor of emergence, the moths should have emerged after 19·00 hours rather than between 15.00 hours and 19.00 hours. However, the records at Kew Observatory show that 29th July was a very dull day, and it is possible that the intensity of the light had greatly decreased before 19.00 hours.

Experiment 18.

The emergence of *Drosophila melanogaster* in the laboratory.

Method.—Observations were made every four hours on the emergence from the pupae of *D. melanogaster* reared on banana and agar in glass jars.

Result.—The emergence of flies during a period of 13 days is given in Table XVI. This table shows that there is a very definite rhythm of emergence with a maximum between 6.00 hours and 14.00 hours. There is no indication of whether this peak is caused by the diurnal rhythm of temperature, or of light and darkness, as the cultures were exposed to both.

TABLE XVI.

Times between observations	22–2	2–6	6–10	10–14	14–18	18–22
Number of emergences	1	30	433	262	71	23

Experiment 19.

The emergence of *D. melanogaster* in the laboratory under conditions of constant darkness.

Method.—A number of cultures was divided into three lots. Lot "A" was put on a table exposed to the light and darkness rhythm of the laboratory. The culture jars in lot "B" were kept in tins from which the lids were removed at 14·00 hours and put on again at 2·00 hours. Lot "C" was kept covered all the time. Observations were made every four hours.

Result.—Table XVII gives the result of this experiment. All three lots show a very definite emergence rhythm, with the maximum emergence from the two lots in the tins ("B" and "C") occurring slightly later than from lot "A."

TABLE XVII.

Times between observations	22–2	2–6	6–10	10–14	14–18	18–22
Number of emergences from "A"	19	87	463	486	109	19
"B"	21	41	180	387	207	51
"C"	10	35	118	248	54	8

IV. DISCUSSION.

Bremer (1926 (5)) states that two groups of factors may cause the emergence rhythm: (1) fixed hereditary factors, and (2) outside influences. The investigation of the latter is the more important and fixed factors need only be considered when outside conditions fail to give a satisfactory explanation of the phenomena observed. Outside factors which cause a rhythm with a diurnal periodicity must themselves show a daily period.

The factors which have a daily periodicity are temperature, light, barometric pressure, relative humidity, electrical potential and electrical conductivity of the air.

Barometric pressure and the electrical potential of the air have a semi-diurnal rhythm with a maximum at 10.00 hours and 22.00 hours and a minimum at 4.00 hours and 15.00 hours. If either of these factors is the cause of the emergence rhythm shown by insects there should be two periods of maximum emergence in each 24 hours. Further, in this country the diurnal rhythm of both barometric pressure and electrical conductivity is completely masked by other changes. A record of the barometric pressure in the constant-temperature room was kept for some time, but no diurnal periodicity was discovered. As the electrical potential is closely connected with the barometric pressure it may be assumed that neither of these factors is the cause of the emergence rhythm.

The relative humidity of the air has a well-defined diurnal rhythm. It is high at 3.00 hours and low at 15.00 hours. This rhythm is connected with the temperature rhythm, and records made in the constant-temperature room show that, although the relative humidity changes, these changes are not rhythmic. Even in the insectary it is doubtful if there is a diurnal rhythm of relative humidity in the cultures, as flour is slow to react to any change. Little information could be obtained about the diurnal rhythm of electrical conductivity of the air as continuous records are not made. However, meteorologists consider that there is no diurnal rhythm of this factor that would have any effect inside a building.

Temperature and light rhythms have the greatest diurnal fluctuation and so are the most important factors to be considered in connection with the emergence rhythm. No external factors show a diurnal rhythm in the constant-temperature room except light and the disturbance caused by people entering during the day. The light factor is eliminated when the cultures are kept in tins. Presumably a disturbance, if it had any effect, would cause immediate emergence, but the maximum emergence was always during the night when there was no disturbing influence.

The emergence rhythm shown by *E. kühniella* Z. in the insectary is very marked. Very few moths emerged during the morning and early afternoon, but they emerged in large numbers during the evening. Graphs I and II show that this emergence rhythm is closely connected with the diurnal rhythm of temperature and that the maximum emergence occurs when the temperature begins to fall. In all experiments showing a fall of temperature there was a corresponding period of maximum emergence of moths. This is clearly seen from Graphs III, IV, V and VI.

When pupae were moved from the insectary to the constant-temperature room the rhythm of emergence was continued. This rhythm is not so marked as when there was a temperature rhythm present and many moths were found to emerge during the morning and afternoon. Even so the maximum emergence still occurs in the evening. Cultures kept in tins in the constant-temperature room could not be affected by any diurnal change of an external factor so that this rhythm must have been acquired by the larvae while in the insectary. The results of Experiment 5 prove that this rhythm of emergence is retained by insects which were moved to the constant-temperature room while still in the larval stage. All the cultures of *E. kühniella* Z. in the insectary had their period of maximum emergence at the same hour, but in the constant-temperature room the peak of emergence in some cultures is before, and in others, later than normal. This suggests that although the insects have a rhythm of emergence impressed on them during the larval and pupal stages they need the regulating effect of an external rhythmic factor to ensure

emergence at the correct time. This suggestion would also explain the emergence of a large number of moths between 2.00 hours and 14.00 hours in the constant-temperature room.

It is unfortunate that moths reared at 27° C. tend to become sterile. This had the effect of reducing the number of emergences from the third generation in Experiment 7. However, the moths were numerous enough to show that the rhythm of emergence was continued in constant conditions for three generations. As no external factor shows a periodicity of 24 hours in the constant-temperature room, *E. kühniella* Z. must possess an inherent emergence rhythm. This is emphasised when it is remembered that many of the parent moths emerged during the morning and early afternoon, and the culture from which some of them emerged showed only a slight rhythm.

The results obtained from Experiment 8 show that it is possible to change the time of maximum emergence by moving the cultures from a low to a high temperature for two hours each day. The changes of temperature in this experiment were very sudden and differ in this respect from the normal diurnal rhythm of temperature. The same result was obtained from Experiments 10 and 11, when the cultures were subjected to a diurnal temperature rhythm with the maximum occurring before the usual time. The maximum emergence always occurred after the temperature began to fall. When the cultures in Experiment 11 were moved to the constant-temperature room the maximum emergence occurred at the same hour of the day as the last fall of temperature which they had experienced. Thus the fall of temperature not only causes an immediate emergence but impresses a rhythm of emergence on the insects which are due to emerge during the next 24 hours. The attempt to induce a rhythm of emergence with a periodicity of more than 24 hours (Experiment 12) failed because of this impressed rhythm, which caused many moths to emerge 24 hours after the last fall in temperature even when the temperature was now rising. This shows that the 24-hour period is a fundamental part of the emergence rhythm, and supports the suggestion that the emergence rhythm of insects reared in the constant-temperature room is caused by an inherited factor and not by any rhythmic external factor.

The attempt to reduce the length of the periodicity of the emergence rhythm was successful. As before, the moths emerged when the temperature fell.

The nature of this inherited factor is difficult to understand, but in some respects it appears to be comparable to the inheritance of such a character as size in a species, which varies about a mean but is easily modified by the environment. The factor governing rhythm appears to have two functions. The first causes the greatest number of moths to emerge in the evening, and it is effective for at least three generations when variable external factors are eliminated. However, this inherited time of maximum emergence can be changed by certain external factors, and the insects can be made to emerge in greatest numbers at any time of the day. The second function is connected with the 24-hour period, and it was impossible to lengthen this period. Since it was possible to decrease the length of the emergence rhythm by means of a 16-hour temperature rhythm, falling temperature, which is the most important external factor controlling emergence, is stronger than the inherited factor.

The results given by the four experiments on the influence of the light factor are somewhat contradictory. It is difficult to understand how light can have an effect on the emergence rhythm of *E. kühniella* Z., as the pupae

are in a cocoon which is covered with flour and which appears to be very opaque. The pupae were not removed from their cocoons in any of the experiments, and in Experiment 17 they were further protected from light by being buried in flour. Experiment 17 is the only one of the four experiments in which all the cultures were subjected to the same temperature rhythm. It is probable that the temperature rhythm was different for the individual lots of cultures in the other three experiments, and this would account for the contradictory results. These experiments suggest that the change of emergence rhythm of *D. alopecuri* found by Barnes (1) was also due to temperature and not to light. Bremer's (5) experiment with "reversed" lighting gave results similar to those obtained from Experiments 14 and 15. This led him to conclude that the reduction of light is an incentive to emergence. It seems, however, more probable that the change of emergence in this experiment was caused by changes of temperature.

Experiment 17 appears to show that light of more than a certain intensity is an inhibitor of emergence. No attempt has been made to discover how long strong light can inhibit emergence, nor what intensity of light is required; but the intensity of light at a distance of 3 feet from a 75-watt electric lamp is not sufficient (Experiment 14).

The two experiments with *D. melanogaster* show that this fly exhibits a diurnal rhythm of emergence. The time of maximum emergence is between 6.00 hours and 14.00 hours. This rhythm is continued in darkness so that probably it is connected with the rhythmic change of temperature.

No attempt has been made to discover how the temperature rhythm causes the emergence rhythm. None of the theories proposed to explain the rhythms shown by other organisms are applicable, and it is impossible to offer an acceptable explanation from the evidence available.

V. Conclusion.

(1) In the insectary *E. kühniella* Z. shows a diurnal rhythm of emergence with a maximum emergence in the evening. This emergence rhythm exhibits a close relationship with the diurnal temperature rhythm. The maximum emergence occurs shortly after the temperature begins to fall.

(2) The emergence rhythm is continued when larvae and pupae are moved into the constant-temperature room. The peak of maximum emergence is not so well defined here, for moths are found to emerge at all times throughout the day. The time of maximum emergence may be earlier or later than in the insectary. This shows that although the rhythm is maintained the influence of an outside factor is needed to maintain the peak at the correct time of day.

(3) The third generation of moths reared in constant conditions still maintained the rhythm of emergence. The rhythm is therefore inherited.

(4) The time of maximum emergence can be changed by changing the time of maximum temperature. The moths always emerge in greatest numbers when the temperature begins to fall. The new time of maximum emergence is maintained in constant conditions.

(5) An emergence rhythm with a periodicity of 16 hours was induced by a temperature rhythm of the same period, but an attempt to induce one with a 36-hour period failed as many moths emerged 24 hours after the last fall of temperature. A 24-hour periodicity seems to be a fundamental part of the emergence rhythm.

(6) When light is above a certain intensity it appears to inhibit emergence.

(7) *D. melanogaster* shows a diurnal emergence rhythm with a maximum emergence between 6.00 hours and 14.00 hours. This rhythm is continued in darkness.

(8) The diurnal rhythm of temperature is the external factor which induces the emergence rhythm of *E. kühniella* Z. Apparently there is also an inherited tendency to have an emergence rhythm with a periodicity of 24 hours.

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